



Biomedical Sciences
University of Missouri

Identifying orexin receptors on hypothalamic neurons activated by hypoxia

Marshall R. Browne, Ruwaida Ben Musa, Jennifer Cornelius-Green,
David D. Kline, and Kevin J. Cummings

Department of Biomedical Sciences, College of Veterinary Medicine
Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO

Introduction

- ❑ The peripheral chemoreflex (PCR) is critical for the adaptive increases in breathing and sympathetic nervous system activity (SNA) in response to hypoxia (Hx). **The PCR is critical for maintaining oxygenation of the brain during Hx.**
- ❑ The PCR is initiated by the carotid chemoreceptors which transmit the afferent signal to the nucleus of the solitary tract (nTS) to activate cardiorespiratory and SNA neural circuits in response to Hx.
- ❑ The hypothalamus helps regulate feeding behavior, body temperature, state of vigilance, autonomic activity, and respiratory control.
- ❑ The paraventricular nucleus (PVN) in the hypothalamus contributes to the ventilatory (HVR) and SNA responses to Hx, in part via corticotropin-releasing hormone (CRH) neurons that project to and help activate nTS neurons in response hypoxia.
- ❑ Our lab recently showed that orexin (Ox), synthesized by hypothalamic neurons that project to the PVN, also contributes to the HVR, but the mechanism(s) are unknown.

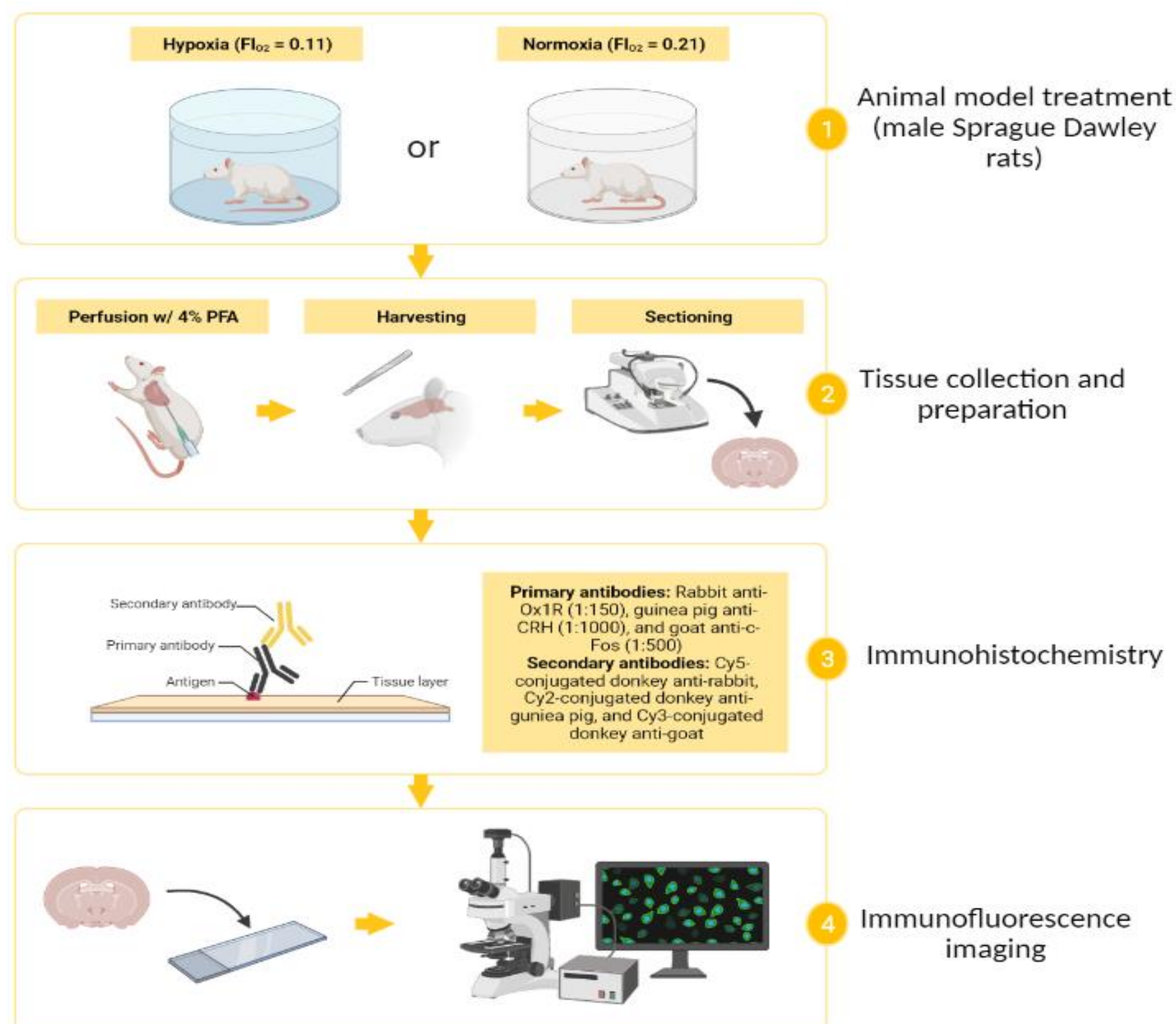
Hypothesis:

- ❑ Orexin receptors are expressed on CRH neurons that are activated by hypoxia and project to the nTS.

Objective

- ❑ Our objective is to resolve whether Ox1R is expressed by CRH neurons in the PVN that are activated by Hx and project to nTS.

Methods



1. Rats were exposed to either normoxia or hypoxia for two hours to induce activation.
2. Their brains were fixed with paraformaldehyde, harvested, and sectioned.
3. Sections were mounted on slides for immunohistochemistry (IHC) to detect c-Fos (marker of cellular activation), CRH, and Ox1R. Primary antibodies against these targets, followed by secondary antibodies labelled with fluorophores, were incubated with the slices from the PVN.
4. Immunofluorescence visualized the Ox1R on activated, CRH neurons.

Orexin receptors present on CRH neurons

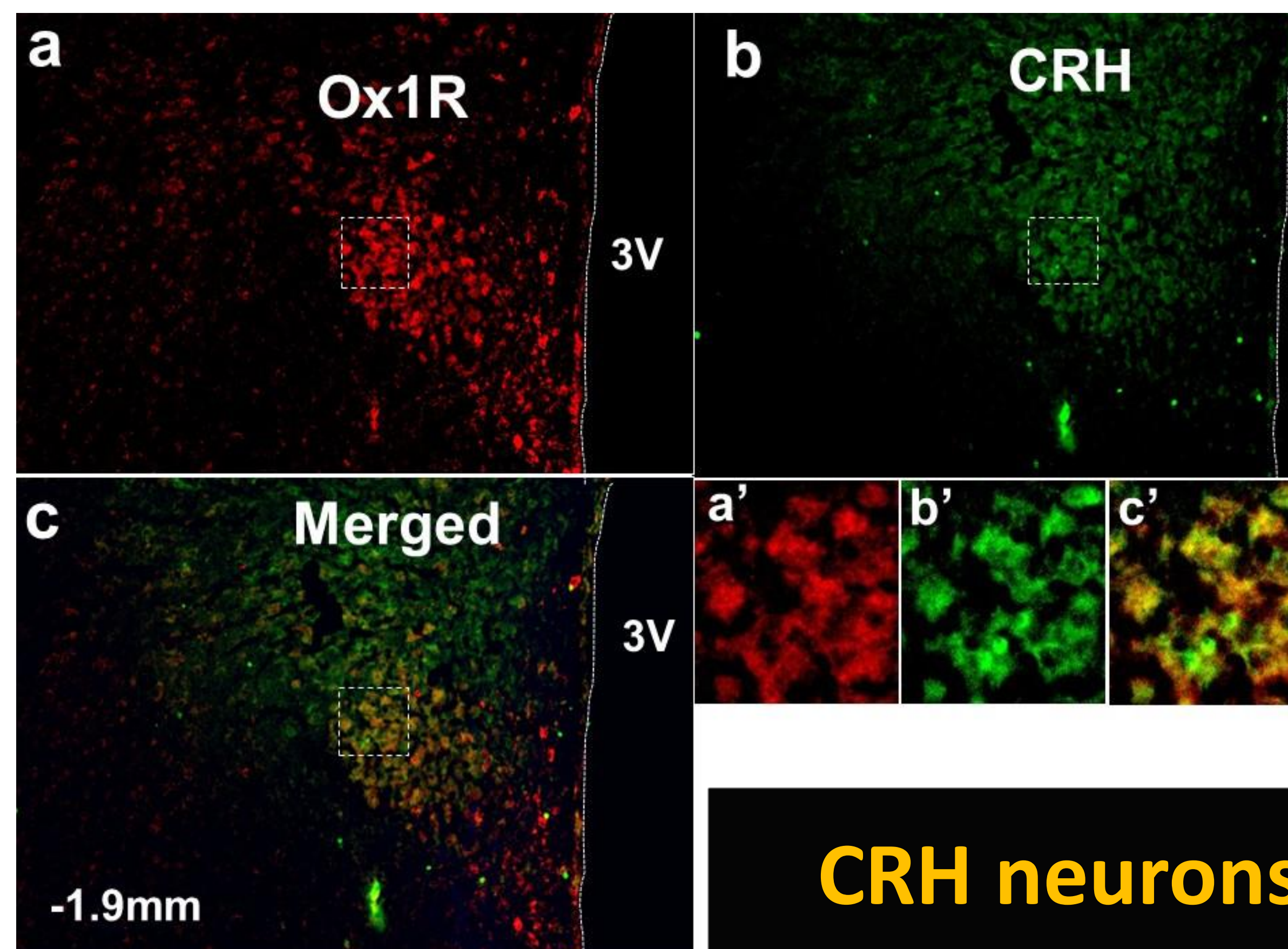
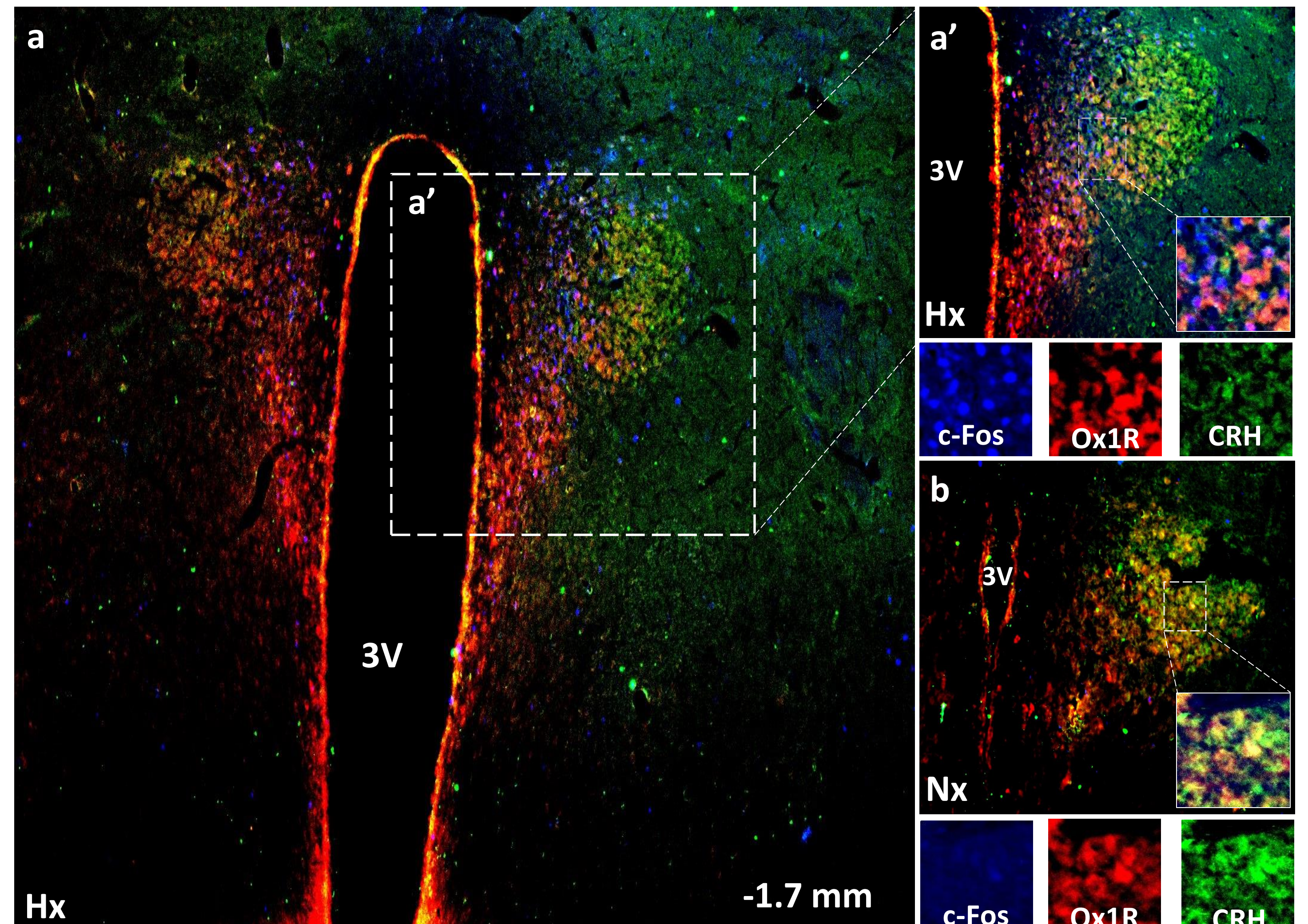


Figure 1. Presence of orexin receptors on CRH neurons in the paraventricular nucleus (PVN). IHC was done on the PVN of rat brains at different levels from the bregma. Images of coronal hypothalamus (-1.9 mm relative to bregma) from male rat exposed to hypoxia ($FI_{O_2} = 11\%$) for 2 hr. Shown is the 3rd ventricle (3V) adjacent to the PVN. (a) Image of orexin 1 receptor (Ox1R). a': higher magnification of Ox1R receptors in PVN. (b) Image of corticotropin-releasing hormone (CRH) neurons. b': higher magnification of CRH neurons in PVN. (c) Merged images of neurons expressing Ox1R (red), CRH (green), and both proteins (orange). c': higher magnification neurons co-labelled for Ox1R and CRH.

CRH neurons are activated by Hx

Figure 2. Comparison of the PVN of rats exposed to normoxia (Nx) or hypoxia (Hx). IHC was done on the PVN of rat brains at different levels from the bregma. Images of coronal hypothalamus (-1.7 mm relative to bregma) from male rats exposed to either hypoxia ($FI_{O_2} = 11\%$) or normoxia ($FI_{O_2} = 21\%$) for 2 hr. Shown is the 3rd ventricle (3V) adjacent to the PVN. (a) Image of merged triple-labeled c-Fos (blue), Ox1R (red), and CRH neurons (green) of rat exposed to hypoxia. a': higher magnification of triple-labeled neurons along with separate images of c-Fos, Ox1R, and CRH neurons. (b) Higher magnification of PVN of rat exposed to normoxia with same triple label as hypoxia along with separate images of c-Fos, Ox1R, and CRH neurons.



- ❑ Orexin receptors (Ox1R) were present on CRH neurons in the PVN (Fig. 1).
- ❑ Activated and inactivated CRH neurons express Ox1R (Fig. 2).

Conclusions

- ❑ These results suggest corticotropin-releasing hormone (CRH) neurons express orexin receptor type 1 (Ox1R).
- ❑ Ox1R activation may facilitate the activation of CRH neurons in response to hypoxia.

Future Directions

- ❑ Improve IHC staining for CRH using antigen retrieval.
- ❑ Determine if Ox1R-expressing CRH neurons that are activated by Hx project to nTS, and whether OxR blockade reduces their activation.

Acknowledgements

- ❑ This project was funded by the National Institute of Health grant R01HL098602 (to KJC).
- ❑ Student support for the VRSP program was provided by Boehringer Ingelheim.